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Blanda et al.

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(54) **AUTOCLAVABLE SUSPENSIONS OF CYCLOSPORIN A FORM 2**

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(51) **Int. Cl.**

A61K 38/13	(2006.01)
A61K 47/38	(2006.01)
A61K 47/32	(2006.01)
A61K 9/00	(2006.01)
A61K 47/36	(2006.01)
C07K 1/30	(2006.01)
C07K 7/64	(2006.01)

(52) **U.S. Cl.**

CPC ***A61K 38/13*** (2013.01); ***A61K 9/0048*** (2013.01); ***A61K 47/32*** (2013.01); ***A61K 47/36*** (2013.01); ***A61K 47/38*** (2013.01); ***C07K 1/306*** (2013.01); ***C07K 7/645*** (2013.01)

(58) **Field of Classification Search**

CPC A61K 38/13; A61K 47/32; A61K 47/38; A61K 47/36; A61K 9/0048; C07K 1/306; C07K 7/645

See application file for complete search history.

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Primary Examiner — Julie Ha
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(57) **ABSTRACT**

Disclosed herein are autoclavable formulations of cyclosporin A Form 2, methods of making such formulations, and methods of treating diseases of the eye with such formulations.

2 Claims, 12 Drawing Sheets

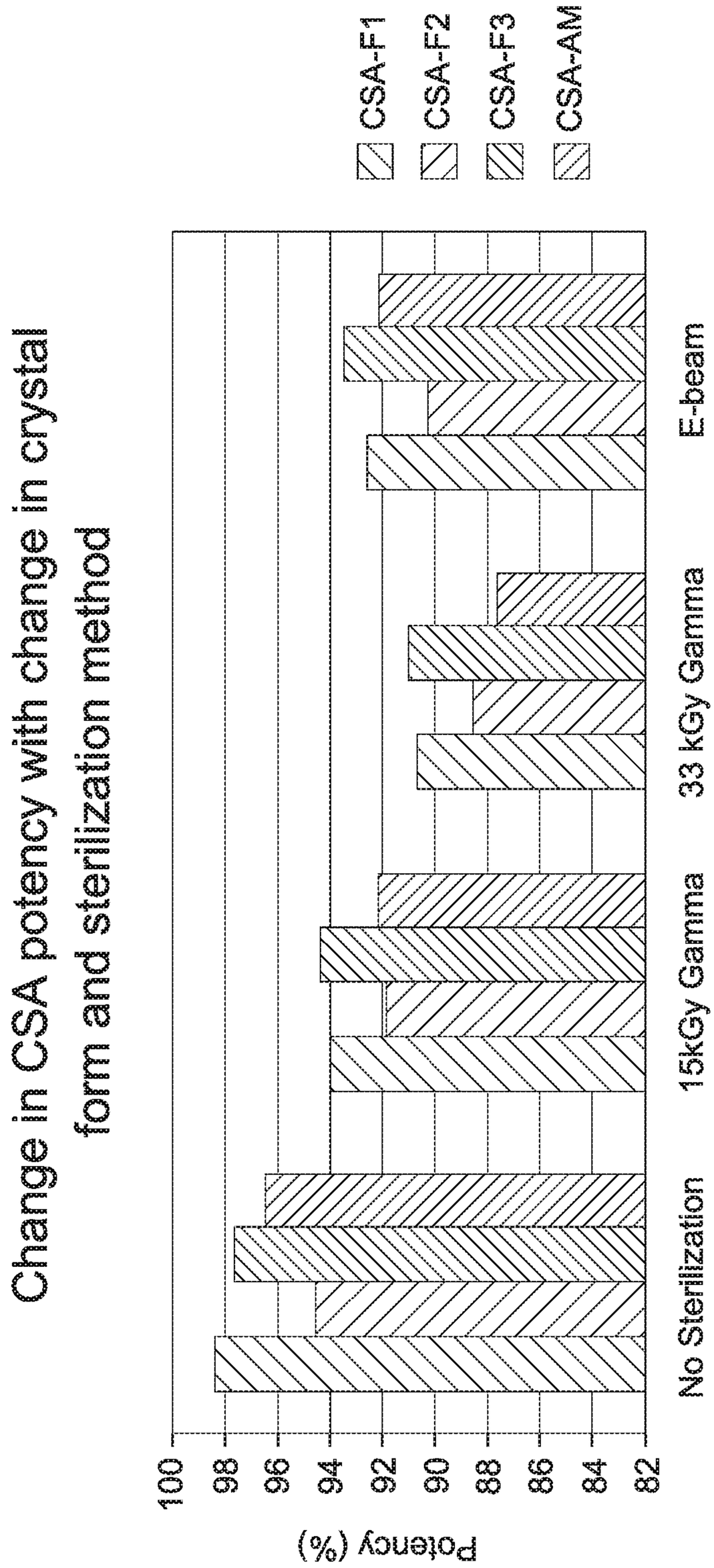

CSA-F1
CSA-F2
CSA-F3
CSA-AM
Change in CSA potency with change in crystal form and sterilization method
Potency (%)
100
98
96
94
92
90
88
86
84
82
No Sterilization
15kGy Gamma
33 kGy Gamma
E-beam
Sterilization Method

FIG. 1

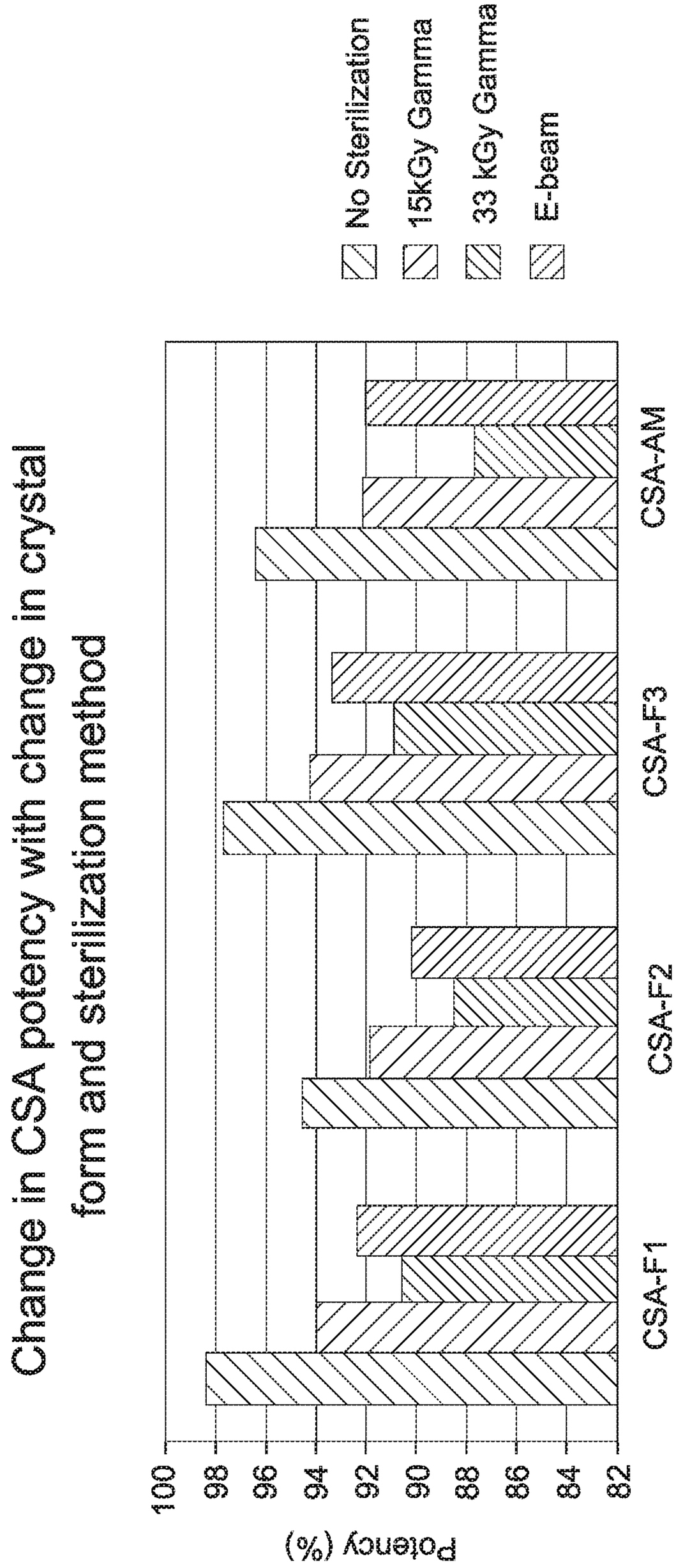

No Sterilization
15kGy Gamma
33 kGy Gamma
E-beam
Change in CSA potency with change in crystal form and sterilization method
CSA-F1
CSA-F2
CSA-F3
CSA-AM
CSA Crystal Form
100
98
96
94
92
90
88
86
84
82
Potency (%)

FIG. 2

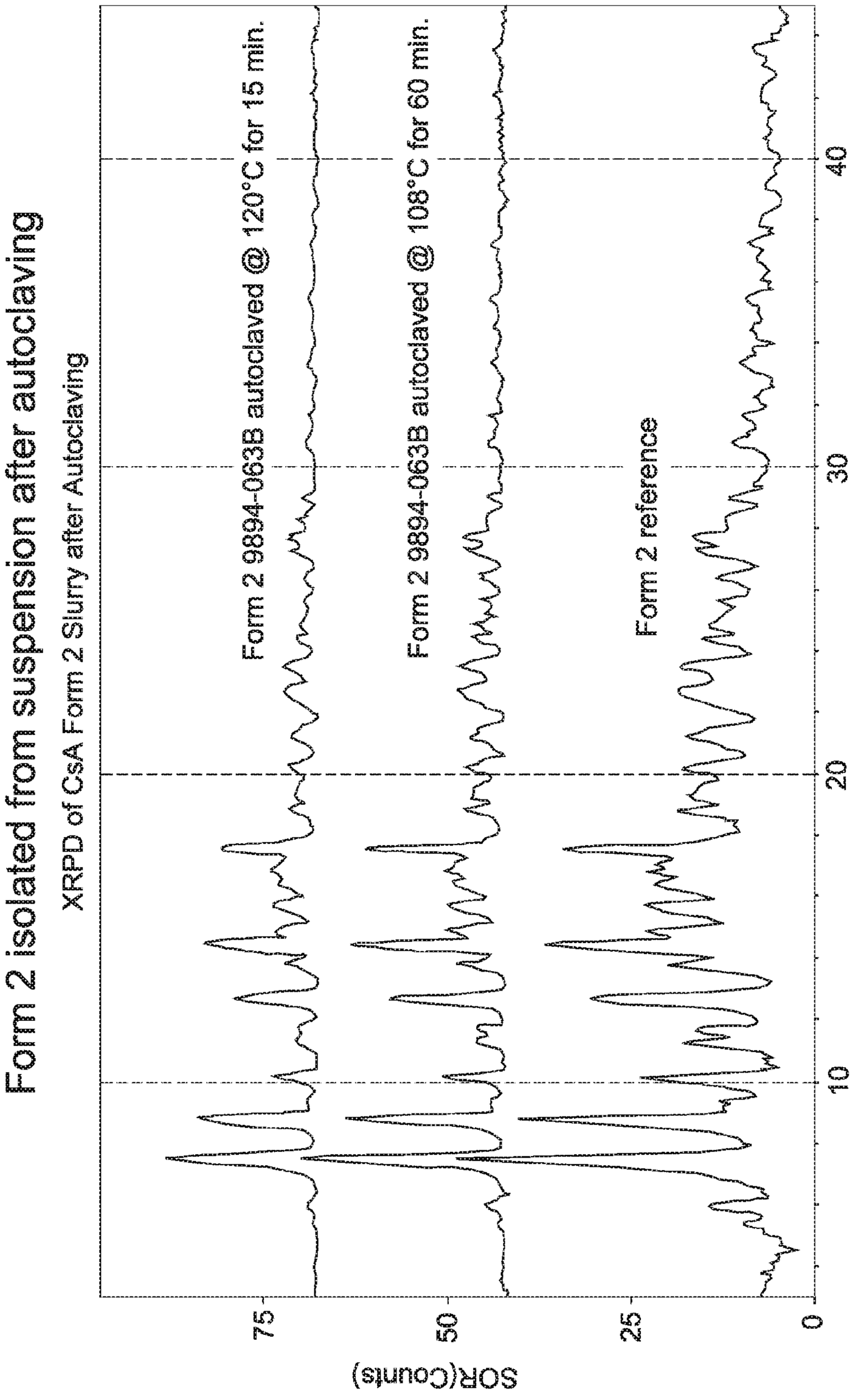

Form 2 isolated from suspension after autoclaving
XRPD of CsA Form 2 Slurry after Autoclaving
Form 2 9894-063B autoclaved @ 120°C for 15 min.
Form 2 9894-063B autoclaved @ 108°C for 60 min.
Form 2 reference
SOR(Counts)
Two-Theta (deg)
0
25
50
75
10
20
30
40

FIG. 3

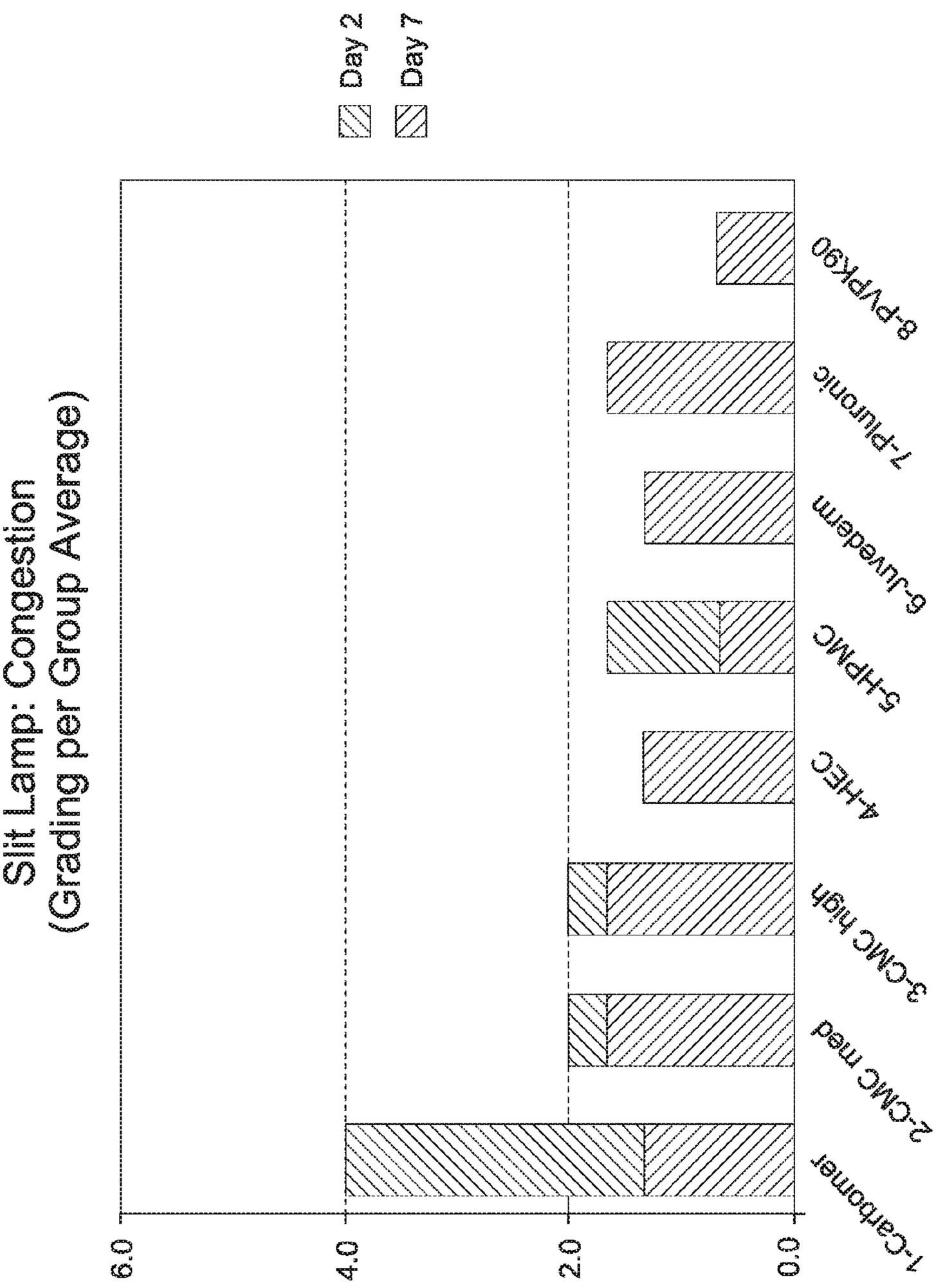

Day 2
Day 7
Slit Lamp: Congestion
(Grading per Group Average)
9.0
4.0
2.0
0.0
1-Carbomer
2-CMC med
3-CMC high
4-HEC
5-HPMC
6-Luvedem
7-Pluronic
8-PVPK90

FIG. 4

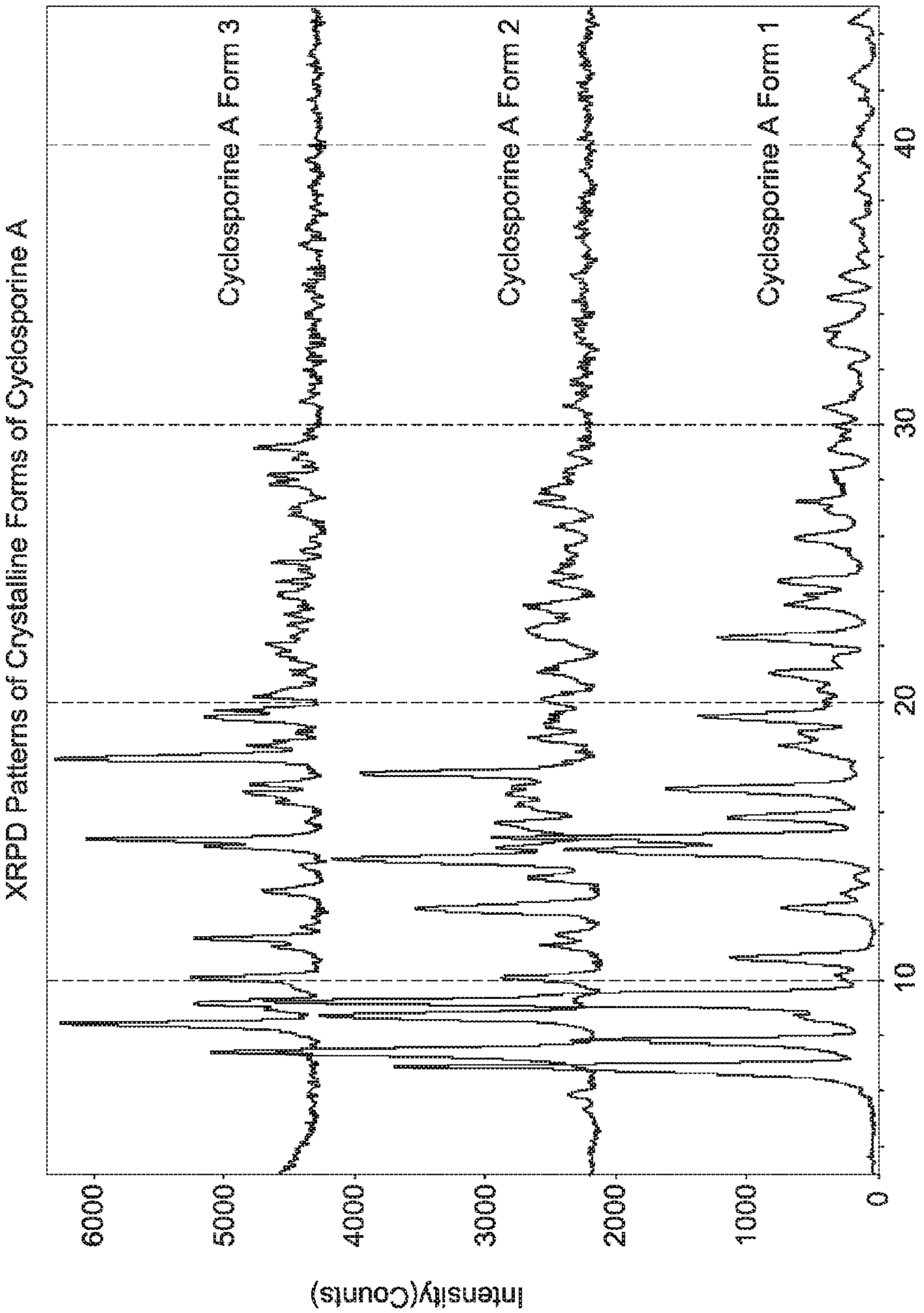

XRPD Patterns of Crystalline Forms of Cyclosporine A
Cyclosporine A Form 3
Cyclosporine A Form 2
Cyclosporine A Form 1
Intensity(Counts)
6000
5000
4000
3000
2000
1000
0
10
20
30
40
Two- Theta (deg)

FIG. 5

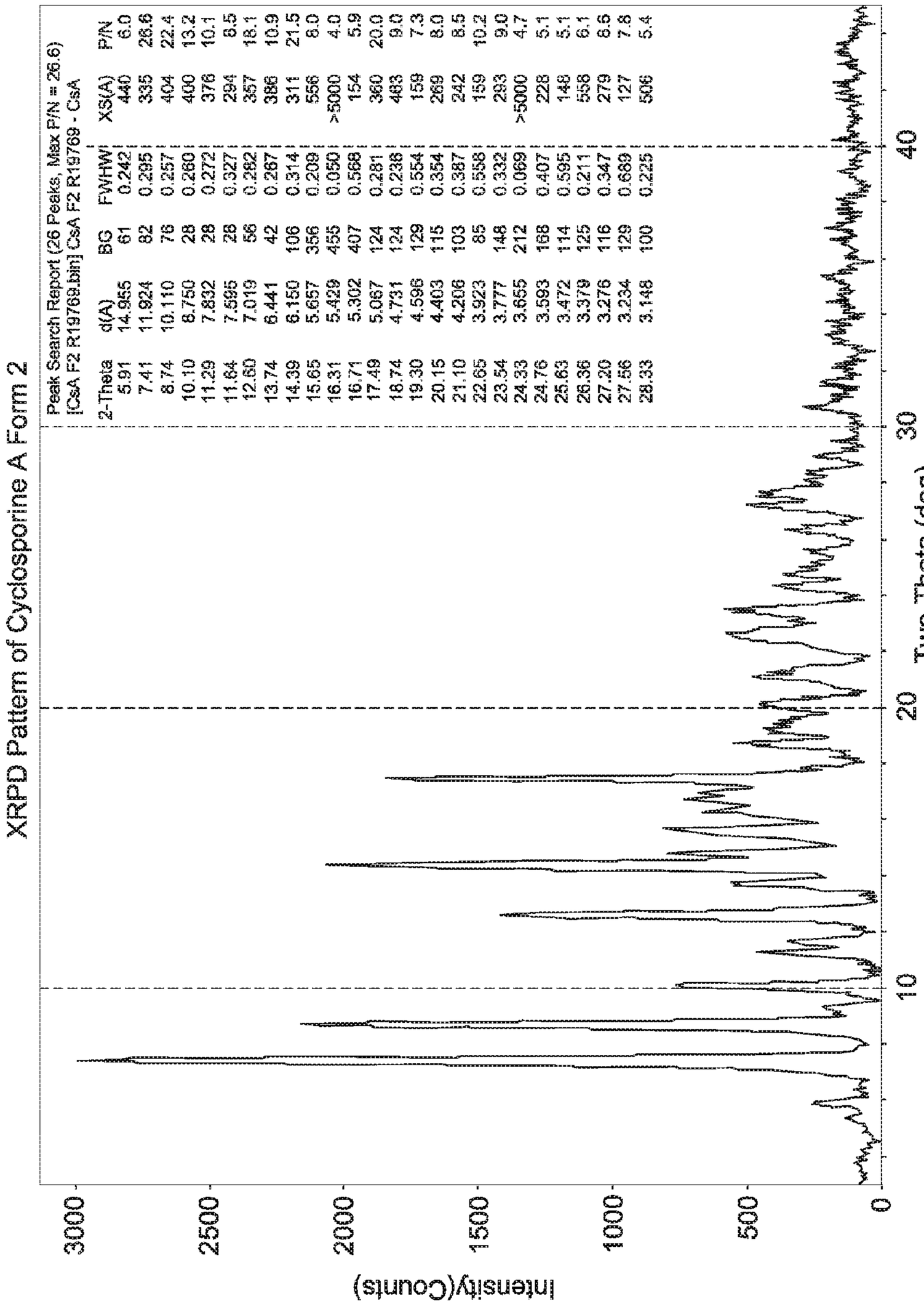

XRPD Pattern of Cyclosporine A Form 2
Peak Search Report (26 Peaks, Max P/N = 26.6)
[CsA F2 R19769.bin] CsA F2 R19769 - CsA
2-Theta d(A) BG FWHW XS(A) P/N
5.91 14.955 61 0.242 440 6.0
7.41 11.924 82 0.295 335 26.6
8.74 10.110 76 0.257 404 22.4
10.10 8.750 28 0.260 400 13.2
11.29 7.832 28 0.272 376 10.1
11.84 7.595 28 0.327 294 8.5
12.60 7.019 56 0.282 357 18.1
13.74 6.441 42 0.267 386 10.9
14.39 6.150 106 0.314 311 21.5
15.65 5.657 356 0.209 556 8.0
16.31 5.429 455 0.050 >5000 4.0
16.71 5.302 407 0.568 154 5.9
17.49 5.067 124 0.281 360 20.0
18.74 4.731 124 0.238 463 9.0
19.30 4.596 129 0.554 159 7.3
20.15 4.403 115 0.354 269 8.0
21.10 4.206 103 0.387 242 8.5
22.65 3.923 85 0.558 159 10.2
23.54 3.777 148 0.332 293 9.0
24.33 3.655 212 0.069 >5000 4.7
24.76 3.593 168 0.407 228 5.1
25.63 3.472 114 0.595 148 5.1
26.36 3.379 125 0.211 558 6.1
27.20 3.276 116 0.347 279 8.5
27.56 3.234 129 0.689 127 7.8
28.33 3.148 100 0.225 506 5.4
Intensity(Counts)
3000
2500
2000
1500
1000
500
0
10
20
30
40
Two-Theta (deg)

FIG. 6

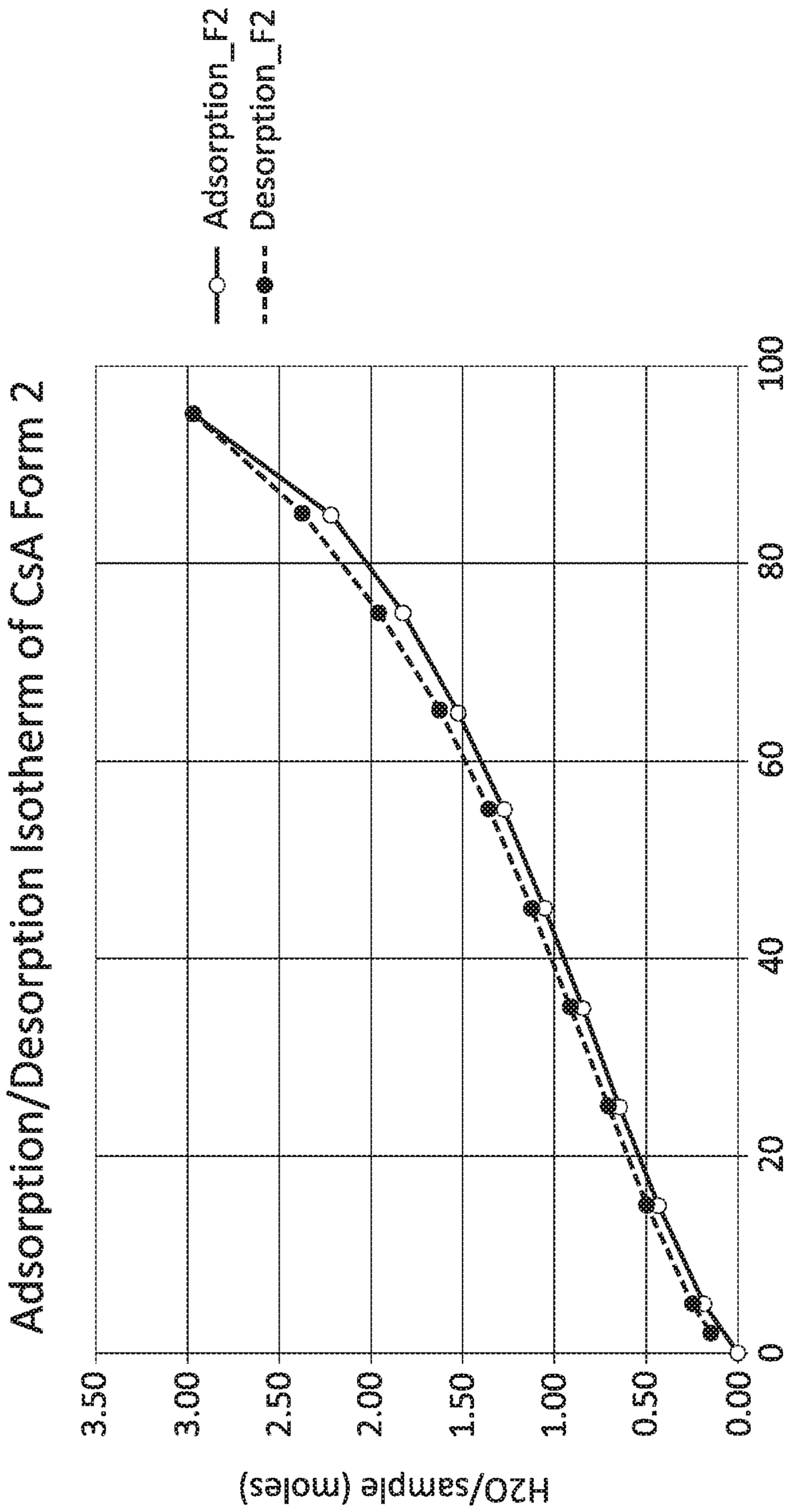

Adsorption_F2
Desorption_F2
Adsorption/Desorption Isotherm of CsA Form 2
%RH
H2O/sample (moles)
0
20
40
60
80
100
0.00
0.50
1.00
1.50
2.00
2.50
3.00
3.50

FIG. 7

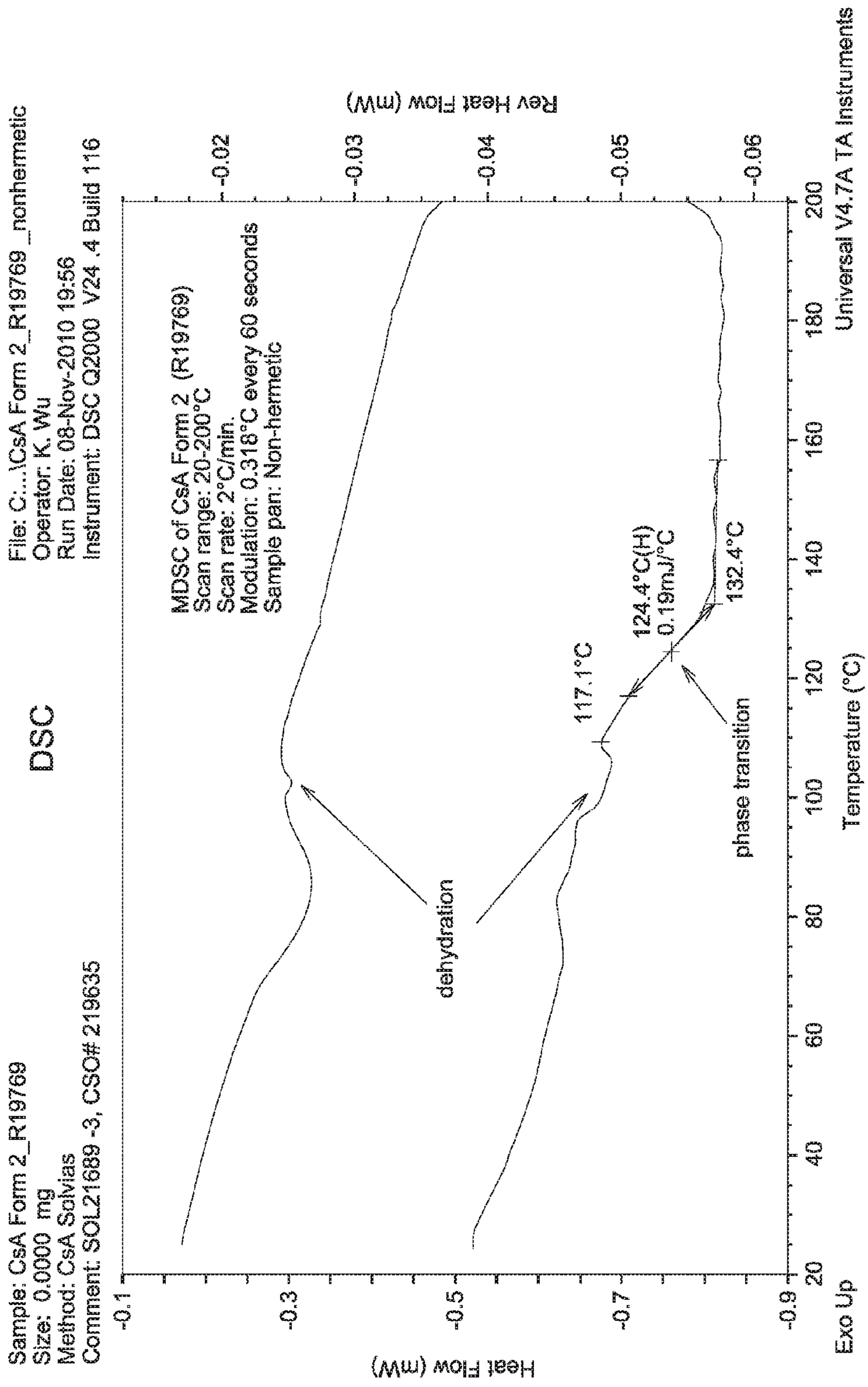

DSC
File: C:...\CsA Form 2_R19769 _nonhermetic
Operator: K. Wu
Run Date: 08-Nov-2010 19:56
Instrument: DSC Q2000 V24 .4 Build 116
Sample: CsA Form 2_R19769
Size: 0.0000 mg
Method: CsA Solvias
Comment: SOL21689 -3, CSO# 219635
MDSC of CsA Form 2 (R19769)
Scan range: 20-200°C
Scan rate: 2°C/min.
Modulation: 0.318°C every 60 seconds
Sample pan: Non-hermetic
dehydration
117.1°C
124.4°C(H)
0.19mJ/°C
132.4°C
phase transition
Rev Heat Flow (mW)
Heat Flow (mW)
Temperature (°C)
Exo Up
Universal V4.7A TA Instruments

FIG. 8

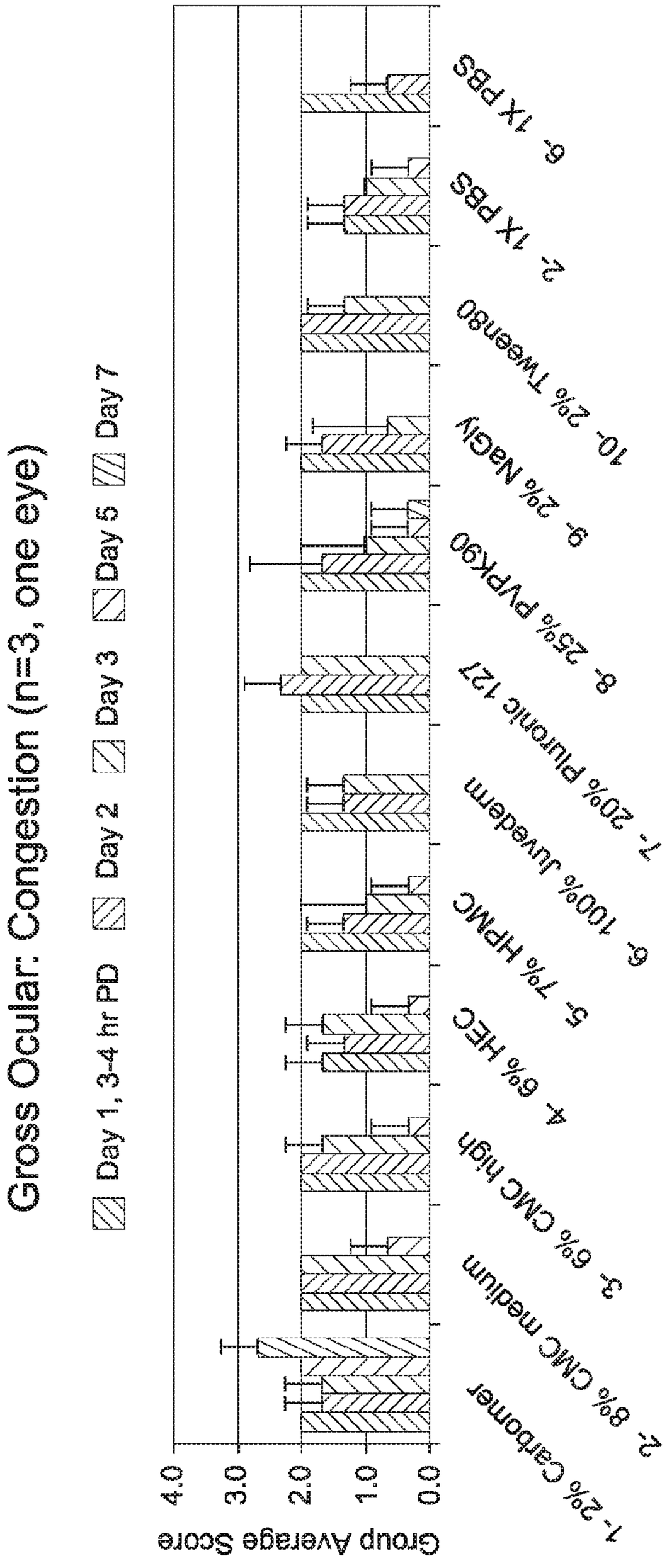

Gross Ocular: Congestion (n=3, one eye)
Day 1, 3-4 hr PD
Day 2
Day 3
Day 5
Day 7
Group Average Score
4.0
3.0
2.0
1.0
0.0
1- 2% Carbomer
2- 8% CMC medium
3- 6% CMC high
4- 6% HEC
5- 7% HPMC
6- 100% Juvederm
7- 20% Pluronic 127
8- 25% PVPK90
9- 2% NaGlY
10- 2% TWeen80
2- 1X PBS
6- 1X PBS
Group Number and Vehicle

FIG. 9

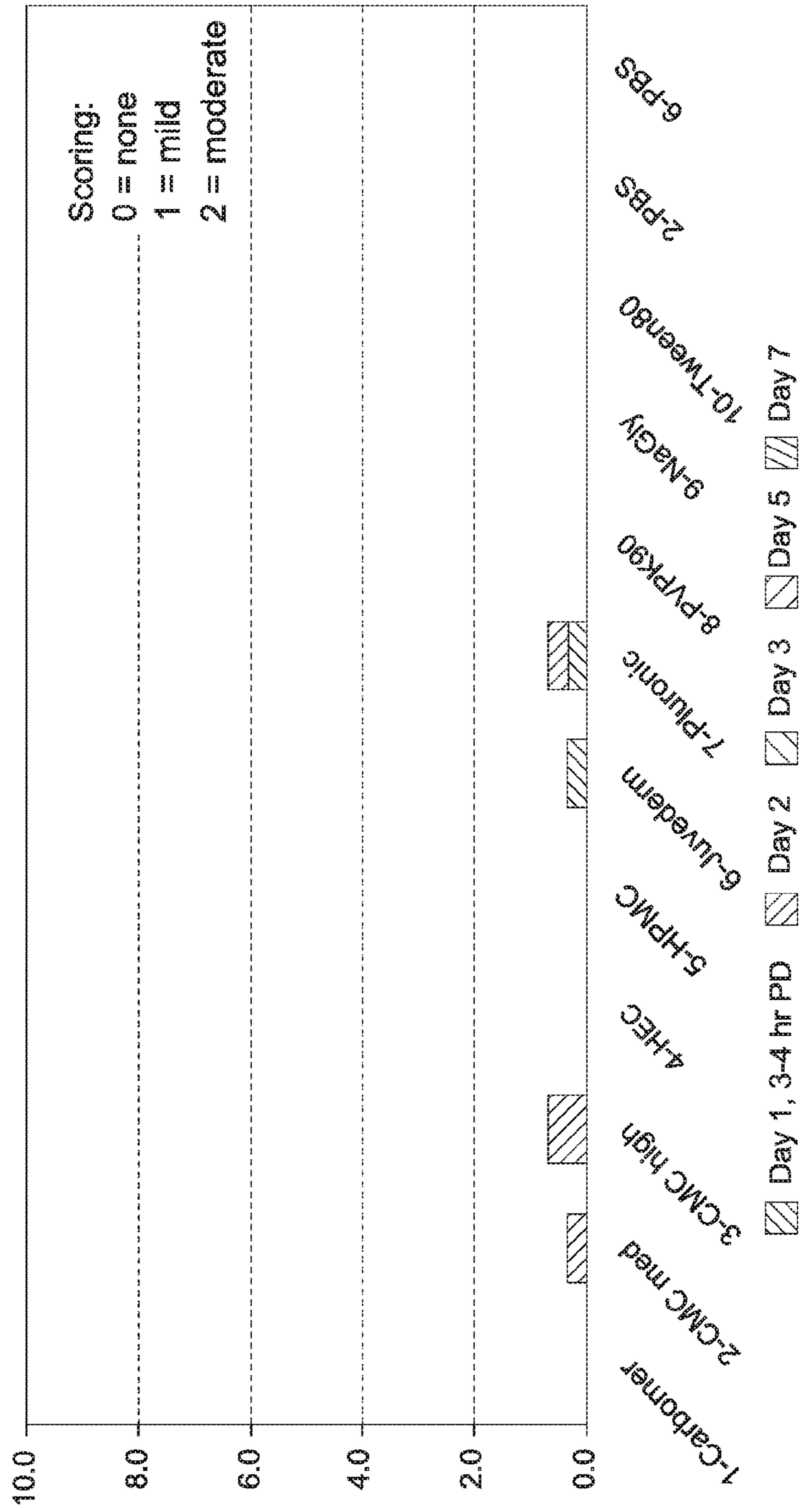

Gross Ocular: Discharge/Tearing
(Grading per Group Average)
Scoring:
0 = none
1 = mild
2 = moderate
10.0
8.0
6.0
4.0
2.0
0.0
1-Carbomer
2-CMC med
3-CMC high
4-HEC
5-HPMC
6-Juvederm
7-Pluronic
8-PVPK90
9-NaGly
10-Tween80
Day 1, 3-4 hr PD
Day 2
Day 3
Day 5
Day 7

FIG. 10

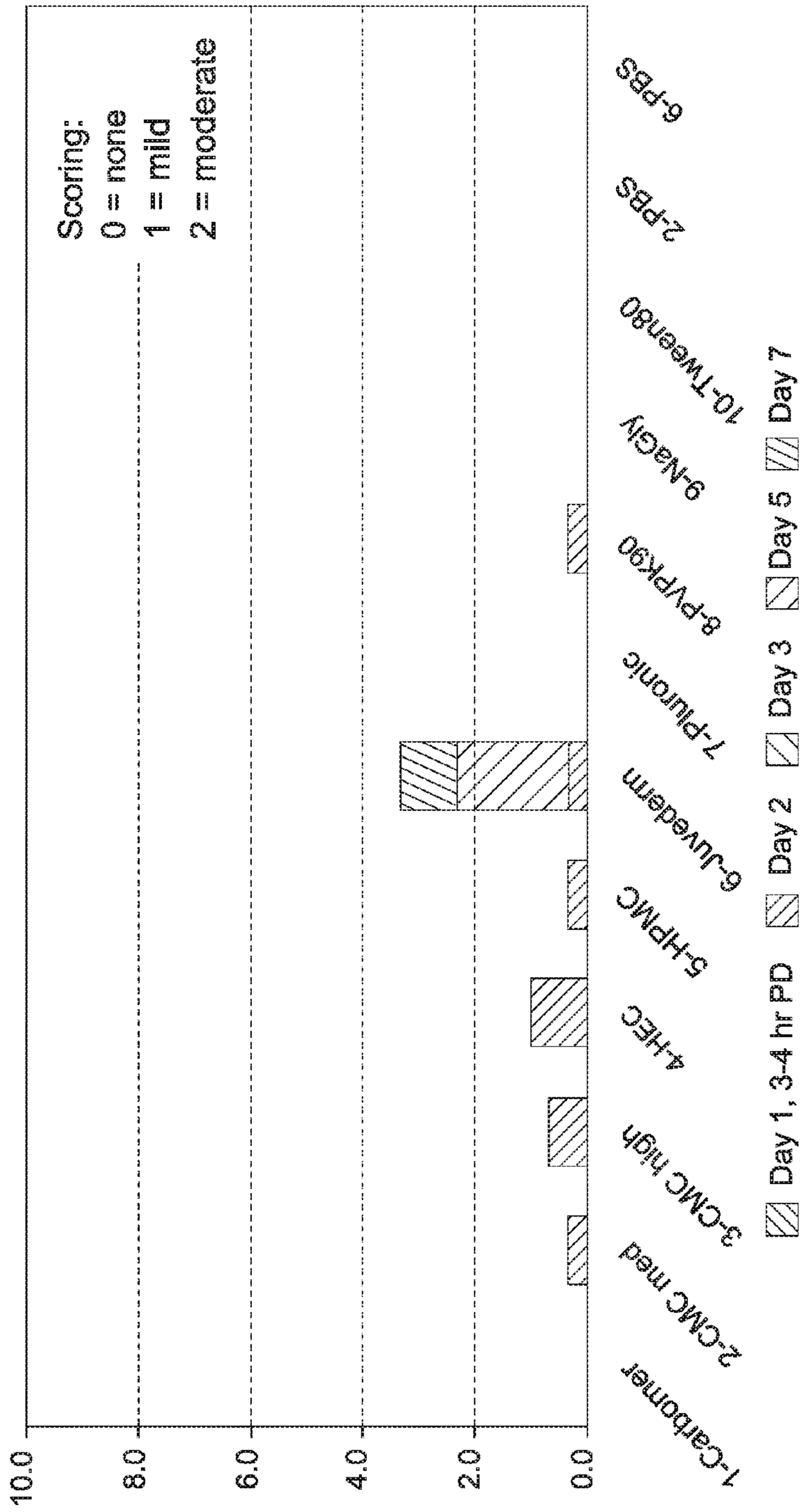

Gross Ocular: Swelling
(Grading per Group Average)
Scoring:
0 = none
1 = mild
2 = moderate
10.0
8.0
6.0
4.0
2.0
0.0
1-Carbomer
2-CMC med
3-CMC high
4-HEC
5-HPMC
6-Juvederm
7-Pluronic
8-PVPK90
9-NaGlv
10-Tween80
Day 1, 3-4 hr PD
Day 2
Day 3
Day 5
Day 7
6-PBS
7-PBS

FIG. 11

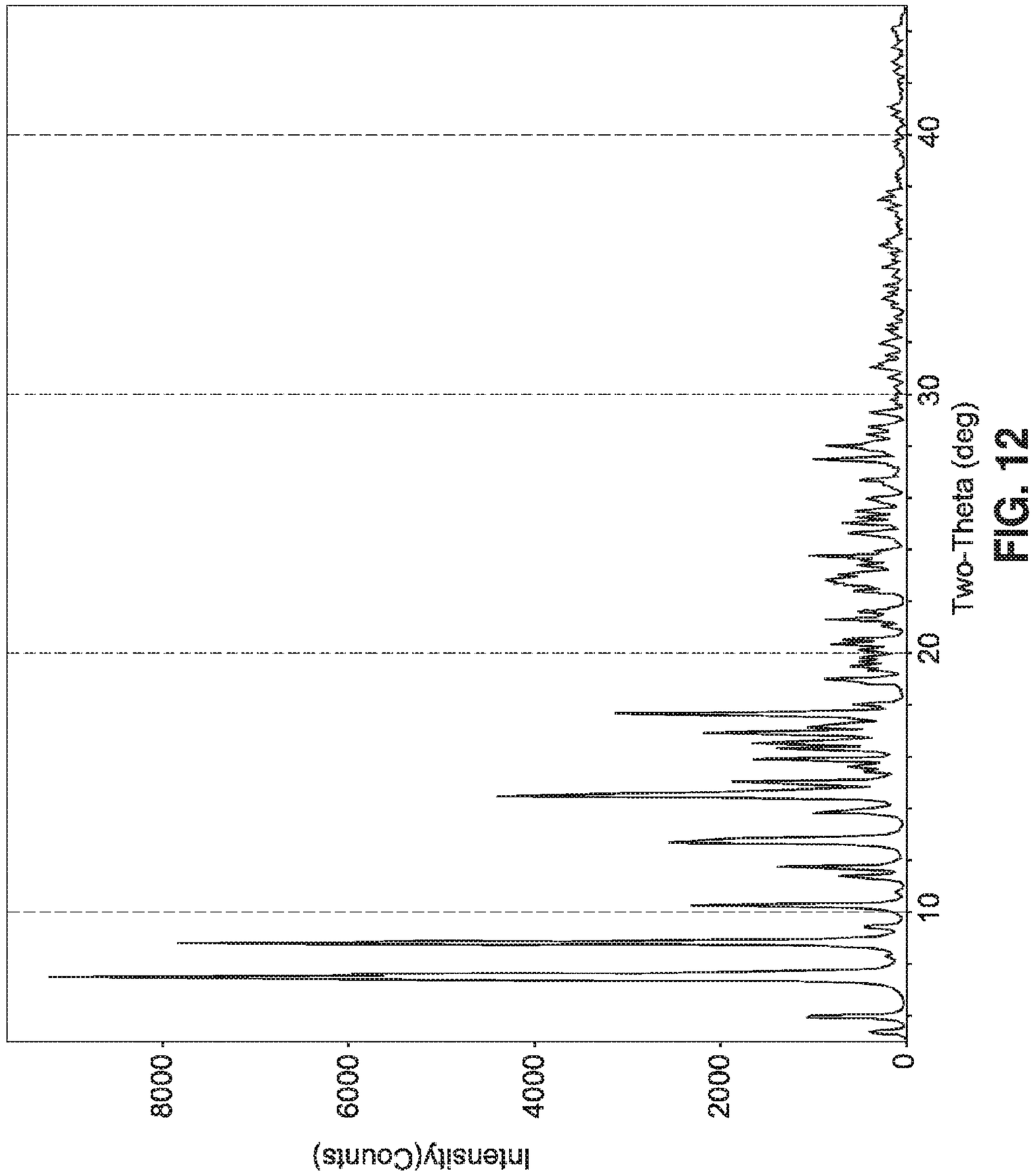

Intensity(Counts)
8000
6000
4000
2000
0
10
20
30
40
Two-Theta (deg)

FIG. 12

AUTOCLAVABLE SUSPENSIONS OF CYCLOSPORIN A FORM 2

CROSS-REFERENCE TO RELATED APPLICATION

This patent application is a divisional of copending U.S. patent application Ser. No. 13/676,362, filed Nov. 14, 2012, now abandoned, which claims priority to U.S. Provisional Patent Application No. 61/559,849, filed Nov. 15, 2011, the entire contents of which are hereby incorporated by reference.

BACKGROUND

Aseptic processing of cyclosporin A suspensions in a hyaluronic acid media (a hydrogel used as a suspending agent), is complicated by the fact that both the drug and the hyaluronic acid need to be pre-sterilized. Pre-sterilized hyaluronic acid is extremely expensive, costing roughly $1 million dollars for a few kilograms (roughly $10,000 per ounce) of sterile raw material. Additionally, in the process of pre-sterilizing cyclosporin A, the drug is degraded upon irradiation, as shown below and in FIGS. **1** and **2**:

Additional levels of degradants need to be qualified in preclinical safety studies. Moreover, a suspension, prepared with only 90-95% of the labeled Cyclosporin A (due to the pre-sterilization process), has a substantial probability of failure to meet regulatory guidelines for shelf-life, since regulatory authorities generally prohibit shelf-lives below 90% of label.

The present invention solves these problems. Disclosed herein are formulations of cyclosporin A, combined with a parenterally-biocompatible suspending agent, which are sterile, exceptionally stable to heat sterilization, and have excellent long-term stability.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. **1** and **2** show change in cyclosporin A potency with change in crystal form and sterilization method.

FIG. **3** shows x-ray powder diffraction pattern data of cyclosporin A Form 2 after autoclaving.

FIG. **4** shows congestion seen on slit lamp examination with eight different formulations.

TABLE 1

Impact of Irradiation on Cyclosporin Stability

Sterilization Mode	Form 1 CsA (Potency and Imp.)	Form 2 CsA (Potency and Imp.)	Form 3 CsA (Potency and Imp.)	Amorph, CsA (Potency and Imp.)
None	98.4% w/w Total Imp: 0.6%	94.6% w/w Total Imp: 0.6%	97.7% w/w Total Imp: 0.8%	96.5% w/w Total Imp: 0.7%
15 kGy Gamma	93.9% w/w % Rel. Change: 4.5% Total Imp: 1.7%	91.8% w/w % Rel. Change: 2.9% Total Imp: 1.8%	94.3% w/w % Rel. Change: 3.6% Total Imp: 1.3%	92.1% w/w % Rel. Change: 4.6% Total Imp: 1.4%
30 kGy Gamma	90.7% w/w % Rel. Change: 7.8% Total Imp: 2.8%	88.5% w/w % Rel. Change: 6.4% Total Imp: 2.4%	91.0% w/w % Rel. Change: 6.9% Total Imp: 2.3%	87.7% w/w % Rel. Change: 9.2% Total Imp: 2.3%
E-Beam	92.6% w/w % Rel. Change: 5.9% Total Imp: 1.5%	90.3% w/w % Rel. Change: 4.6% Total Imp: 1.7%	93.4% w/w % Rel. Change: 4.5% Total Imp: 1.6%	92.0% w/w % Rel. Change: 4.7% Total Imp: 1.3%

Cooling the cyclosporin during irradiation does not significantly improve the results, as shown in Table 2, below:

TABLE 2

Impact on Cyclosporin Stability after irradiation under Cold Conditions

Sterilization Mode	Form 1 CsA (Potency and Imp.)	Form 2 CsA (Potency and Imp.)	Form 3 CsA (Potency and Imp.)	Amorph, CsA (Potency and Imp.)
None	99.4% w/w Total Imp: 0.7%	97.6% w/w Total Imp: 0.5%	98.4% w/w Total Imp: 0.7%	96.5% w/w Total Imp: 0.7%
Cold E-beam	94.6% w/w % Rel. Change: 4.8% Total Imp: 1.5%	91.1% w/w % Rel. Change: 6.7% Total Imp: 1.5%	94.6% w/w % Rel. Change: 3.9% Total Imp: 1.8%	92.3% w/w % Rel. Change: 4.4% Total Imp: 1.3%
Regular E-Beam (from Previous Study) % Relative Change in Potency on Sterilization	% Rel. Change: 5.9% Total Imp: 1.5%	% Rel. Change: 4.6% Total Imp: 1.7%	% Rel. Change: 4.5% Total Imp: 1.6%	% Rel. Change: 4.7% Total Imp: 1.3%

FIG. **5** depicts characteristic X-ray powder diffraction (XRPD) patterns of CsA in a new crystalline form (desig

nated as Form 2 herein), tetragonal form (designated as Form 1 herein), and orthorhombic form (designated as Form 3 herein).

FIG. **6** depicts the XRPD diffractogram of CsA crystalline Form 2.

FIG. **7** depicts the water sorption/desorption profile of CsA Form 2.

FIG. **8** depicts MDSC analysis of CsA Form 2 recovered from 0.04% formulation with 1% PS80.

FIG. **9** shows gross ocular congestion after an injection of 100 ul of CMC, HEC, HPMC, Pluronic and PVP in phosphate buffered saline was administered subconjunctivally to New Zealand white rabbits. The rabbits were observed for seven days.

FIG. **10** shows gross ocular discharge in the experiment described in FIG. **9**.

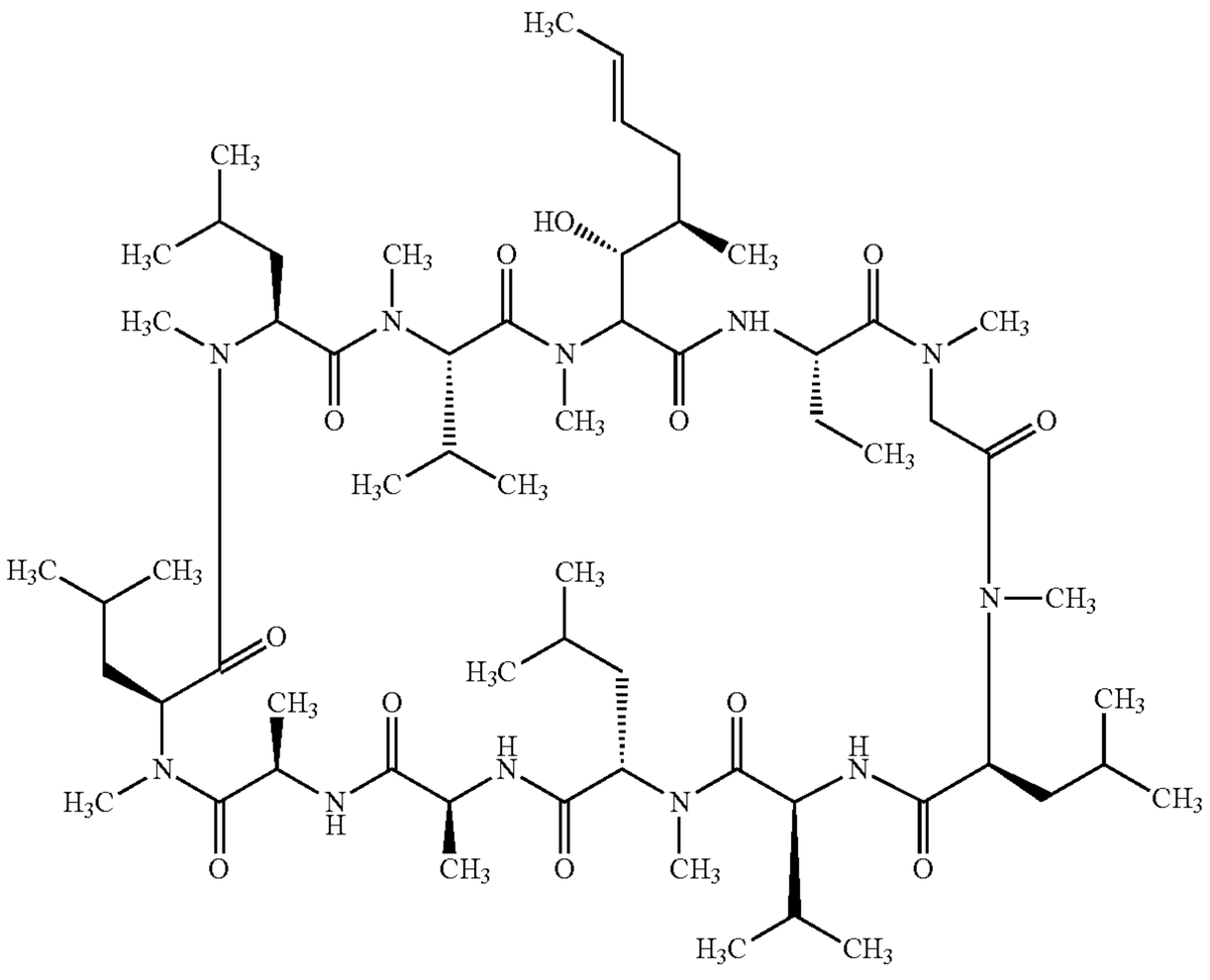
FIG. **11** shows gross ocular swelling in the experiment described in FIG. **9**.

FIG. **12** shows the simulated XRPD pattern of cyclosporin A forms.

DETAILED DESCRIPTION

Cyclosporin A

Cyclosporin A (CsA) is a cyclic peptide having the following chemical structure:



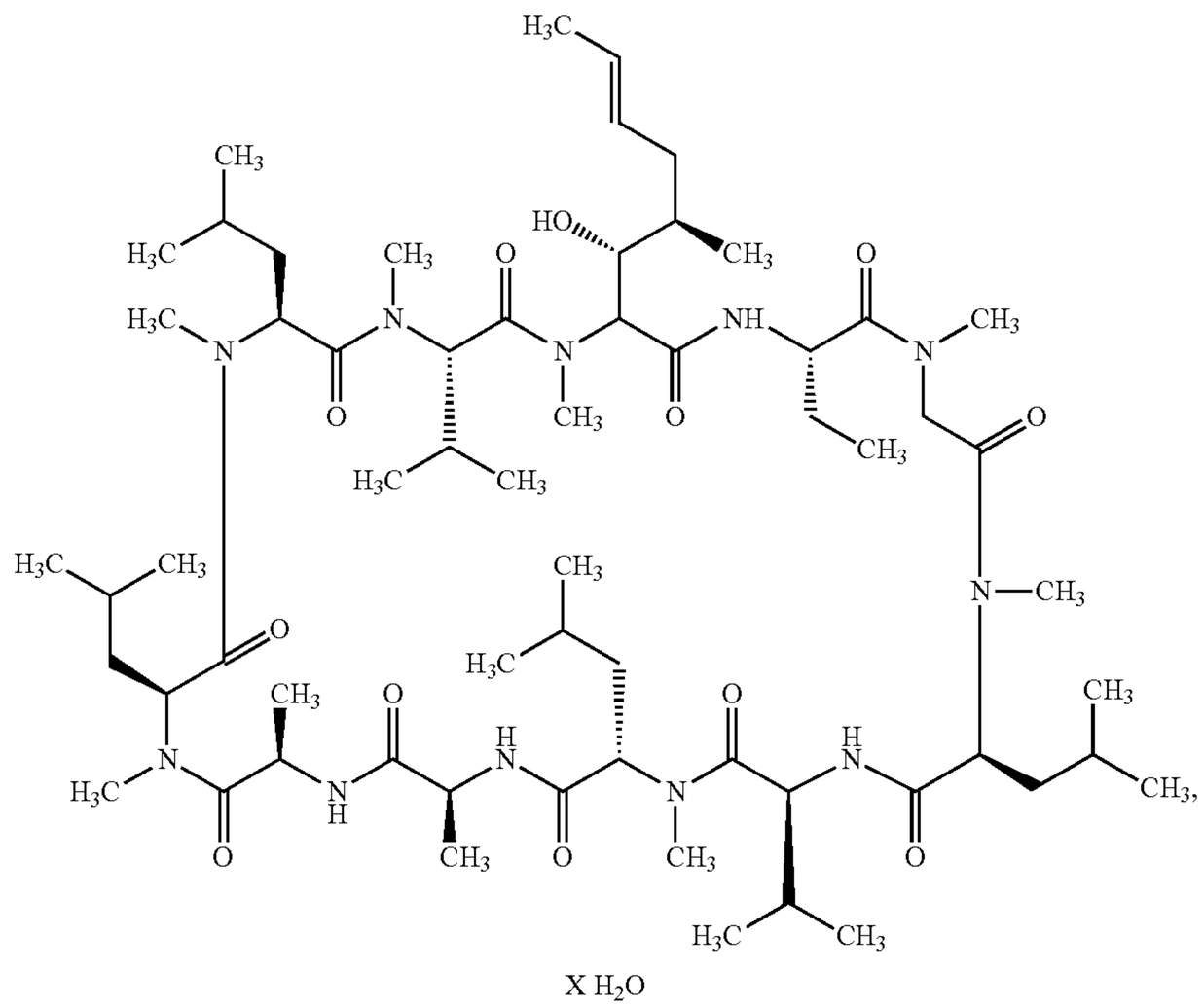
Its chemical name is cyclo[(E)-(2S,3R,4R)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl]-2-aminobutyryl-N-methylglycyl-N-methyl-L-leucyl-L-valyl-N-methyl-L-leucyl-L-alanyl-D-alanyl-N-methyl-L-leucyl-N-methyl-L-leucyl-N-methyl-L-valyl]. It is also known by the names cyclosporin, cyclosporine A, ciclosporin, and ciclosporin A. It is the active ingredient in Restasis® (Allergan, Inc., Irvine, Calif.), an emulsion comprising 0.05% (w/v) cyclosporin. Restasis® is approved in the United States to increase tear production in patients whose tear production is presumed to be suppressed due to ocular inflammation associated with keratoconjunctivitis sicca.

Cyclosporin A Form 2

Cyclosporin A is known to exist in an amorphous form, liquid crystal form, tetragonal crystalline form (form 1), and an orthorhombic form (form 3). A new crystalline form, cyclosporin A Form 2, has recently been discovered.

The XRPD pattern of CsA Form 2 differs significantly from the tetragonal form and orthorhombic form (FIG. **1**). The major crystalline peaks for CsA form 2 appear at (26) when scanned by an X-ray diffractometer with X-ray source as Cu Kα radiation, A=1.54 Å, at 30 kV/15 mA: 7.5, 8.8, 10.2, 11.3, 12.7, 13.8, 14.5, 15.6 and 17.5 (d-spacing in crystal lattice at about 11.8, 10.0, 8.7, 7.8, 7.0, 6.4, 6.1, 5.6 and 5.1 Å, respectively, FIG. **2**). These major peaks are defined as those being unique to Form 2 relative to the orthorhombic or tetragonal forms; as well as, peaks having an intensity greater than 5 times the background.

In one embodiment, the new crystalline form (Form 2) of CsA is a nonstoichiometric hydrate of Cyclosporin A. In another embodiment, the crystalline Form 2 is represented by the formula:



X H_2O

wherein X is the number of molecules of water and varies from 0 to 3. In one embodiment, X in the above formula is 2.

Form 2 appears to be a kinetically stable form of CsA in aqueous suspensions. Suspensions containing Form 2 show no conversion to other known polymorphic or pseudomorphic forms upon storage. It has been found that Form 1 and the amorphous form convert to Form 2 in the presence of water.

The single crystal structure of the hydrate form of CsA Form 2 has been determined and the crystal structure parameters are listed in Table 2. These results indicate that Form 2 is unique compared to other known crystalline forms of cyclosporin A.

TABLE 1

Crystal data and data collection parameters of crystal structure solution of CsA Form 2.

formula	$C_{62}H_{115}N_{35}O_{14}$
formula weight	1236.67
space group	P 2_1 2_1 2_1 (No. 19)
a (Å)	12.6390 (5)
b (Å)	19.7582 (8)
c (Å)	29.588 (2)
volume (Å^3)	7383.8 (7)
Z	4
d_{calc} (g cm^{-3})	1.114
crystal dimensions (mm)	0.27 × 0.18 × 0.12
temperature (K)	150
radiation (wavelength in Å)	Cu K_2 (1.54184)
monochromator	confocal optics
linear abs coef (mm^{-1})	0.840
absorption correction applied	empirical[a]
transmission factors (min, max)	0.80, 0.93
diffractometer	Rigaku RAPID-II
h, k, l range	−13 to 13 −21 to 21 −32 to 21
2θ range (deg)	5.38-115.00
mosaicity (deg)	1.31
programs used	SHELXTL
F_{exo}	2704.0

TABLE 1-continued

Crystal data and data collection parameters of crystal structure solution of CsA Form 2.

weighting	$1/[\sigma^2(Fo^2) + (0.0845P)^2 + 0.0000P]$ where $P = (Fo^2 + 2Fc^2)/3$
data collected	37360
unique data	9964
R_{exo}	0.077
data used in refinement	9964
cutoff used in R-factor calculations	$Fe^2 > 2.0s(Fe_a^2)$
data with I > 2.0s(I)	6597
number of variables	834
targest shift/esd in final cycle	0.00
$R(F_a)$	0.061
$R_*(F_a^2)$	0.145
goodness of fit	1.037
absolute structure determination	Flack parameter[a] (0.0(3))

The asymmetric unit of this CsA Form 2 contains one cyclosporin A molecule and two water molecules. It is possible that any small molecule that can hydrogen bond to water could play the role of space filler, which would give a range of potential structures running from the orthorhombic dihydrate to distorted monoclinic dihydrate The XRPD pattern calculated from the single-crystal structure is shown in FIG. **12** and it matches the experimental pattern shown in FIG. **2**. These matching patterns further corroborate that Form 2 is a unique and pure crystalline form of cyclosporin A.

Without wishing to be bound by theory, thermogravimetric analysis combined with KF titration and vapor sorption desorption analysis (VSA) suggest that CsA Form 2 is a non-stoichiometric hydrate of CsA. The vapor sorption analysis of Cyclosporin Form 2 indicates that water content in the new crystal form reversibly varies with relative humidity as shown in FIG. **7**. Similar to the tetragonal form, the new CsA form undergoes a phase transition to a liquid crystal or amorphous form at 124.4° C. prior to melting as indicated by the modulated differential calorimetric (MDSC) analysis (FIG. **8**).

Cyclosporin A Form 2 may be obtained by suspending amorphous 0.05% cyclosporin A (w/v) in 1% Polysorbate 80, heating the solution to 65° C., holding it at that temperature for 24 hours, and then recovering the precipitate by vacuum filtration. One can then use the cyclosporin A Form 2 thus obtained to generate additional amounts, using Cyclosporin A Form 2 as a seed crystal; in this method, one suspends about 30 g cyclosporin A in a solution of 900 ml water containing 1% (w/v) Polysorbate 80, heats the solution to 65° C., and then seeds it with 0.2 g of cyclosporin A Form 2 at a temperature of 52° C. The solution is then stirred for about 22 hours at a temperature of between about 61° C. and 65° C., and then recovers the precipitate that results.

Further details regarding CsA Form 2 may be found in U.S. patent application Ser. No. 13/480,710, the entire contents of which are incorporated by reference herein.

Heat-Stable, Heat-Sterilized Suspensions of Cyclosporin A Form 2

Compositions of the invention are ophthalmically acceptable suspensions of Cyclosporin A form 2. By "ophthalmically acceptable," the inventors mean that the suspensions are formulated in such a way as to be non-irritating when administered to the eye of a mammal, such as a human.

The suspensions of the invention comprise cyclosporin A form 2 and a vehicle comprising a suspending agent such as hyaluronic acid, a cellulose, polyvinylpyrrolidone (PVP), Pluronic® copolymers based on ethylene oxide and propylene oxide, and Carbopol® polymers.

In one embodiment, the suspension comprises cyclosporin A Form 2 at a concentration of about 0.001% to about 10% (w/v). In one embodiment, the suspension comprises cyclosporin A form 2 at a concentration of about 0.001% (w/v) to about 0.01%, about 0.001% (w/v) to about 0.04% (w/v), about 0.001% (w/v) to about 0.03% (w/v), about 0.001% (w/v) to about 0.02% (w/v), or about 0.001% (w/v) to about 0.01% (w/v). In another embodiment, the suspension comprises cyclosporin A form 2 at a concentration of about 0.01% (w/v) to about 0.05%, about 0.01% (w/v) to about 0.04% (w/v), about 0.01% (w/v) to about 0.03% (w/v), about 0.01% (w/v) to about 0.02% (w/v), or about 0.01% (w/v) to about 0.01% (w/v). In another embodiment, the suspension comprises cyclosporin A form 2 at a concentration of about 0.01% (w/v) to about 0.1%, about 0.1% (w/v) to about 0.5% (w/v), about 0.01% (w/v) to about 1% (w/v), or about 1% (w/v) to about 10%.

For example, the suspensions may comprise about 0.001% (w/v), about 0.002% (w/v), about 0.003% (w/v), about 0.004% (w/v), about 0.005% (w/v), about 0.006% (w/v), about 0.007% (w/v), about 0.008% (w/v), about 0.009% (w/v), about 0.01% (w/v), about 0.015% (w/v), about 0.02% (w/v), about 0.025% (w/v), about 0.03% (w/v), about 0.035% (w/v), about 0.04% (w/v), about 0.045% (w/v), about 0.05% (w/v), about 0.055% (w/v), about 0.06% (w/v), about 0.065% (w/v), about 0.07% (w/v), about 0.075% (w/v), about 0.08% (w/v), about 0.085% (w/v), about 0.09% (w/v), about 0.095% (w/v), about 0.1% (w/v), about 0.15% (w/v), about 0.2% (w/v), about 0.25% (w/v), about 0.3% (w/v), about 0.35% (w/v), about 0.4% (w/v), about 0.45% (w/v), about 0.5% (w/v), about 0.55% (w/v), about 0.6% (w/v), about 0.65% (w/v), about 0.7% (w/v), about 0.75% (w/v), about 0.8% (w/v), about 0.85% (w/v), about 0.9% (w/v), about 0.95% (w/v), or about 1.0% (w/v) cyclosporin A form 2.

Examples are provided in Table 3, below:

TABLE 3

Autoclavable suspensions of cyclosporin A Form 2.

Formulation	CsA (Crystal form)	CsA (%)	Gelling Agent (Type)	Gelling Agent (%)	Autoclave Conditions (Temp (° C.)/min)
1	2	20	CMC	5	121/10
2	3	20	CMC	3	121/10
3	NA	0	Carbopol Ultrez 10	1.5	121/15
4	NA	0	Carbopol Ultrez 10	2.0	121/15
5	NA	0	Carbopol Ultrez 10	2.5	121/15
6	NA	0	Carbopol Ultrez 10	1.0	121/15
7	NA	0	Carbopol Ultrez 10	4.0	121/15
8	2	5	CMC	3	121/15
9	2	5	CMC	2	121/15
10	2	20	CMC	10	121/15
11	2	0	CMC	10	121/15
12	2	5	HPMC	3	121/15
13	2	5	HPMC	6	121/15
14	2	20	HPMC	6	121/15
15	2	20	HPMC	10	121/15
16	2	5	HPMC	6	121/15
17	2	20	HPMC	3	121/15
18	2	5	HPMC	3	121/15
19	2	20	HPMC	3	121/15
20	2	10	HPMC	4.5	121/15
21	2	10	HPMC	4.5	121/15
22	2	10	HEC	3	121/15
23	2	10	HEC	3	121/15
24	2	30	HEC	1	121/15
25	2	10	HA	3.5	121/15*
26	2	10	HA	2.5	121/15
27	2	30	HEC	1	121/15
28	2	30	HA	1	121/15*
29	2	10	HA	2.5	121/15
30	2	10	HA	3.5	121/15
31	2	10	HA	4.5	121/15
32	2	30	HA	3.0	121/15
33	2	20	HA	1.5	121/15
34	2	20	HA	2.5	121/15
35	2	20	HA	3.5	121/15
36	2	10	HA	4	121/15, 121/30, and 123/15
37	2	10	HA	4	121/15, 121/30, and 123/15
38	2	10	HA	4	121/15, 121/30, and 123/15
39	2	35	HA	1	121/15*
40	2	5	HA	3.5	121/15*
41	2	10	HA	3.5	121/15*
42	2	20	HA	2.0	121/15*
43	2	20	HA	2.0	121/15*
44	2	10	HA	3.5	121/15*
45	2	10	HA	3.5	121/15*
46	2	25	N/A	0	120/15
47	2	25	N/A	0	118/20
48	2	25	N/A	0	120/12
HEC1	2	5	HEC	5	121/15
HEC2	2	20	HEC	5	121/15
HEC3	2	5	HEC	2	121/15
HEC4	2	20	HEC	2	121/15
HEC5	2	5	HEC	5	121/15
HEC6	2	20	HEC	5	121/15
HEC7	2	5	HEC	2	121/15
HEC8	2	20	HEC	2	121/15
HEC9	2	10	HEC	3	121/15
PVP1	2	10	PVP	25	121/15
PVP2	2	10	PVP	25	121/15

TABLE 3-continued

Autoclavable suspensions of cyclosporin A Form 2.

Formulation	CsA (Crystal form)	CsA (%)	Gelling Agent (Type)	Gelling Agent (%)	Autoclave Conditions (Temp (° C.)/min)
PVP3	2	10	PVP	15	121/15
PVP4	2	10	PVP	15	121/15
PVP5	2	25	PVP	25	121/15
PVP6	2	25	PVP	25	121/15
PVP7	2	25	PVP	15	121/15
PVP8	2	25	PVP	15	121/15
PVP9	2	10	PVP	25	121/15
PVP10	2	25	PVP	25	121/15

CsA = cyclosporin A.
CMC = carboxymethyl cellulose.
HPMC = hydroxypropyl methyl cellulose.
HEC = hydroxyethyl cellulose.
HA = hyaluronic acid.
PVP = polyvinylpyrrolidone.
*= slurry autoclaved prior to addition of gelling agent.

Methods of Preparation

Suspensions of the invention contain cyclosporin A Form 2 and a suspending agent. In another embodiment, the suspension also contains one or more of water, buffer, and salt, in sufficient quantities to provide a biocompatible formulation. By "biocompatible," the inventors mean that the suspension is appropriate for administration to the eye (for example, by parenteral administration).

The formulations of the invention may be manufactured by using either a heat-sterilized slurry of Form 2 cyclosporin mixed aseptically with a sterile parenterally-biocompatible suspending agent and other excipient; or by combining Form 2 cyclosporin with a parenterally-biocompatible suspending agent and other excipients and heat sterilizing the entire formulation.

These methods address various important problems with cyclosporin formulation: 1) solid cyclosporin cannot be pre-sterilized by irradiation without significant drug degradation and formation of degradation products; 2) sterile filtration is also not feasible because the formulation is a suspension; and 3) terminal sterilization by heat will decrease gel viscosity. Also, in one embodiment, the final viscosity of the drug formulation is sufficiently high to keep the cyclosporin suspended throughout the product's shelf-life. In another embodiment, the viscosity is sufficiently low to permit the final formulation to flow through a narrow gauge syringe, such as a 22, 23, 24, 25, or 26 gauge needle or narrower. In still another embodiment, the formulation is sufficiently high to keep the cyclosporin suspended throughout the product's shelf-life, and also sufficiently low to permit the final formulation to flow through a syringe with a 22, 23, 24, 25, or 26 gauge needle or narrower.

Methods 1 and 2, below, use hyaluronic acid as the suspending agent but, other suitable suspending agents may be substituted.

It should be noted that sterile hyaluronic acid is very expensive and that method 2 provides a unique method of sterilization, which allows the use of non-sterile hyaluronic acid by heat-reducing the polymer to the correct molecular weight range, so that it reaches the target viscosity range. Method 2, therefore, requires precision manufacturing, where each new lot of hyaluronic acid may shift to a different viscosity range, under identical manufacturing conditions. Consequently, in order to assure the correct viscosity range is reached in every commercial batch, the heat cycle will need to be adaptive—that is—adjusted according to a set of guidelines and experiments on the raw material lot prior to manufacture of the drug product.

Furthermore, it should be noted that Method 2 prepares all steps of the formulation in a single vessel. These two methods allow for the rapid production of the drug product and consequently, have substantial value in saving one day or more of valuable manufacturing time over Method 1.

These methods depend on the inventors' surprising discovery that cyclosporin A Form 2 may be autoclaved and still retain its potency and stability. Other forms of cyclosporin—amorphous, Form 1 and Form 3—cannot be autoclaved, without unacceptable loss of drug substance from the suspension.

Method 1—Aqueous Slurry Method

The appropriate amount of cyclosporin A Form 2 is suspended and mixed in phosphate buffered saline solution and the slurry is heat sterilized by autoclave. In an aseptic environment, the appropriate amount of pre-sterilized hyaluronic acid is added to the sterile cyclosporin slurry, is mixed, and then dissolved. The drug product is brought to volume with sterile water for injection. The final product has a viscosity in the correct range to create a long-term stable suspension, while allowing the final formulation to flow through a syringe fitted with a narrow-gauge needle, such as 25 gauge needle or narrower.

Method 2—Single Vessel Method

An excess of non-sterile hyaluronic acid is dissolved in phosphate buffered saline solution. Cyclosporin A Form 2 is suspended and mixed. The resulting suspension formulation is heat-sterilized by autoclave (using an "adaptive" heat cycle), at the appropriate temperature and for the appropriate amount of time, to both sterilize the formulation and bring the viscosity into the desired range.

For parenteral formulations, it may be desirable to achieve a viscosity that is sufficiently high to keep the cyclosporin suspended throughout the product's shelf-life, and also sufficiently low to permit the final formulation to flow through a syringe with a 22, 23, 24, 25, or 26 gauge needle or narrower. While hydrogel solutions are generally recognized as safe for topical use, very few have been used for parenteral administration, and none have been demonstrated to be safely injected through a 25 gauge needle (or narrower) into subconjunctival tissue at high hydrogel concentrations. A high concentration of suspending agent (up to 25%) is necessary in order to maintain the suspendability of the 5-40% cyclosporin parenteral formulations described herein. In one embodiment, parenteral formulations for use in subconjunctival tissue are (1) injectable through a narrow-gauge needle, such as 25 gauge or narrower, in order to minimize tissue damage by the needle, to allow for quick healing of the needle entry-point, and to limit the back-flow of the injected formulation; (2) sterile; (3) biocompatible; and (4) sufficiently viscous to maintain suspendability throughout the shelf-life of the formulation and to prevent tissue reflux out of the subconjunctival space. In such formulations viscosity is sufficiently high to retain long-term suspendability of the drug but sufficiently low to allow the entire formulation to readily pass through a narrow gauge needle.

In one embodiment of the invention, the formulations have a very high viscosity (e.g., ≥100,000 cps) yet may still able to be injected out of syringe through a narrow-gauge needle. The following table gives examples of such formulations.

	Formulation					
	5% CsA, 3.5% HA (10203X) Viscosity: TBD		10% CsA, 3.5% HA (10204X) Viscosity: 1,300,000 cps		20% CsA, 2.0% HA (10205X) Viscosity: 700,000 cps	
Needle size and type	BD Precision Glide 27 G × 0.5" Needle	TSK Steriject 27 G × 0.5" UTW (Ultra Thin Wall) Needle	BD Precision Glide 27 G × 0.5" Needle	TSK Steriject 27 G × 0.5" UTW (Ultra Thin Wall) Needle	BD Precision-Glide 27 G × 0.5" Needle	TSK Steriject 27 G × 0.5" UTW (Ultra Thin Wall) Needle
Injectabiltiy	✓	✓	✓	✓	✓	✓

Methods of Treatment

Compositions of the invention may be used to treat any condition of the eye which is known to be amenable to topical treatment with cyclosporin A (such as with Restasis®) at the concentrations stated here. For example, compositions of the invention may be used to treat patients suffering from dry eye, to treat blepharitis and meibomian gland disease, to restore corneal sensitivity that has been impaired due to refractive surgery on the eye, to treat allergic conjunctivitis and atopic and vernal keratoconjunctivitis, and to treat pterygium, conjunctival and corneal inflammation, keratoconjunctivitis, graft versus host disease, post-transplant glaucoma, corneal transplants, mycotic keratitis, Thygeson's superficial punctate keratitis, uveitis, and Theodore's superior limbic keratoconjunctivitis, among other conditions.

The International Dry Eye Workshop (DEWS) defines dry eye as "a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface, accompanied by increased osmolarity of the tear film and inflammation of the ocular surface." It includes those conditions, such as keratoconjunctivitis sicca, that are caused by tear deficiency or excessive evaporation of tears.

Blepharitis is a chronic disorder producing inflammation of the anterior and posterior lid margin, with involvement of skin and its related structures (hairs and sebaceous glands), the mucocutaneous junction, and the meibomian glands. It can also affect the conjunctiva, tear film, and the corneal surface in advanced stages and may be associated with dry eye. Blepharitis is commonly classified into anterior or posterior blepharitis, with anterior affecting the lash bearing region of the lids, and posterior primarily affecting the meibomian gland orifices.

Meibomian gland disease most often occurs as one of three forms: primary meibomitis, secondary meibomitis, and meibomian seborrhea. Meibomian seborrhea is characterized by excessive meibomian secretion in the absence of inflammation (hypersecretory meibomian gland disease). Primary meibomitis, by contrast, is distinguished by stagnant and inspissated meibomian secretions (obstructive hypersecretory meibomian gland disease). Secondary meibomitis represents a localized inflammatory response in which the meibomian glands are secondarily inflamed in a spotty fashion from an anterior lid margin blepharitis.

Impaired corneal sensitivity often occurs after refractive surgery, such as photorefractive keratectomy, laser assisted sub-epithelium keratomileusis (LASEK), EPI-LASEK, customized transepithelial non-contact ablation, or other procedures in which the corneal nerves are severed. Impaired corneal sensitivity may also occur after viral infection, such as by HSV-1, HSV-2, and VZV viruses. Patients with impaired corneal sensitivity often complain that their eyes feel dry, even though tear production and evaporation may be normal, suggesting that "dryness" in such patients is actually a form of corneal neuropathy that results when corneal nerves are severed by surgery or inflamed after viral infection.

Allergic conjunctivitis is an inflammation of the conjunctiva resulting from hypersensitivity to one or more allergens. It may be acute, intermittent, or chronic. It occurs seasonally, that is, at only certain time of the year, or it occurs perennially, that is, chronically throughout the year. Symptoms of seasonal and perennial allergic conjunctivitis include, in addition to inflammation of the conjunctiva, lacrimation, tearing, conjunctival vascular dilation, itching, papillary hyperplasia, chemosis, eyelid edema, and discharge from the eye. The discharge may form a crust over the eyes after a night's sleep.

Atopic keratoconjunctivitis is a chronic, severe form of allergic conjunctivitis that often leads to visual impairment. Symptoms include itching, burning, pain, redness, foreign body sensation, light sensitivity and blurry vision. There is often a discharge, especially on awakening from a night's sleep; the discharge may be stringy, ropy, and mucoid. The lower conjunctiva is often more prominently affected than the upper conjunctiva. The conjunctiva may range from pale, edematous, and featureless to having the characteristics of advanced disease, including papillary hypertrophy, subepithelial fibrosis, formix fornix foreshortening, trichiasis, entropion, and madarosis. In some patients the disease progresses to punctate epithelial erosions, corneal neovascularization, and other features of keratopathy which may impair vision. There is typically goblet cell proliferation in the conjunctiva, epithelial pseudotubular formation, and an increased number of degranulating eosinophils and mast cells in the epithelium. CD25+T lymphocytes, macrophages, and dendritic cells (HLA-DR+, HLA-CD1+) are significantly elevated in the substantia propria.

Like atopic keratoconjunctivitis, vernal keratoconjunctivitis is a severe form of allergic conjunctivitis, but it tends to affect the upper conjunctiva more prominently than the lower. It occurs in two forms. In the palpebral form, square, hard, flattened, closely packed papillae are present; in the bulbar (limbal) form, the circumcorneal conjunctiva becomes hypertrophied and grayish. Both forms are often

accompanied by a mucoid discharge. Corneal epithelium loss may occur, accompanied by pain and photophobia, as may central corneal plaques and Trantas' dots.

EXAMPLES

The invention is further illustrated by the following examples.

When the inventors autoclaved aqueous suspensions of cyclosporin A, the drug particles aggregated, making the product unacceptable. Additionally, the inventors found that hyaluronic acid also degrades upon autoclaving, causing a marked drop in viscosity. Lower viscosity, in turn, reduces the suspendability of the drug particles and causes them to settle. Formulations having drug particles in suspension that too rapidly settle, or irreversibly settle, may be useful for laboratory tests, but are not commercially viable.

The inventors explored formulations of four cyclosporin A polymorphic forms, the amorphous form, the tetragonal crystalline form (form 1), the orthorhombic form (form 3), and cyclosporin A Form 2.

A suspension of form 1 converts to the amorphous form and aggregates upon autoclaving; clumping of the cyclosporin is also observed. Consequently, neither form 1 nor the amorphous form is suitable for autoclave stabilization. Furthermore, an autoclaved suspension of F3 in water lost 11-28% of its potency during autoclaving (Table 4); this, too, is unacceptable. In contrast, a suspension of Form 2 in water was quite stable to autoclaving, resisting degradation when compared to a pre-sterilization control. X-ray analysis of filtered solid from the Form 2 formulation also confirms that Form 2 is polymorphically stable to autoclaving (FIG. **3**). These latter two findings are extremely surprising, considering the lack of either chemical or polymorphic stability of the other three forms.

The inventors explored the autoclavability of a series of concentrated solutions of various polymers (no drug) which, when loaded in a syringe, will flow through a narrow-gauge needle (25 gauge or narrower). The polymers evaluated were as follows: crosslinked hyaluronic acid (Juvederm®), carbomer, carboxymethylcellulose-medium molecular weight, carboxymethylcellulose-high molecular weight, hydroxyethylcellulose, hydroxypropylcellulose, Pluronic F127 and polyvinylpyrrolidone K90. All of these are readily available from commercial suppliers.

One hundred microliters of each of the autoclaved solutions was injected into rabbit conjunctiva, in order to evaluate the propensity for causing inflammation. Those polymers producing an inflammatory reaction were eliminated from consideration (FIG. **4**, carbomer, both CMC's, and HPMC were eliminated). Additionally, Juvederm® was eliminated because it formed a long-lasting bleb which, in humans, might cause irritation as the eyelid moves over the site of injection. Both HPMC and Pluronic separated from the solution during/after autoclaving and consequently were also eliminated. Of the commercially viable hydrogels, only HEC and PVP demonstrated that they produced no inflammation in rabbit conjunctiva after autoclaving. These two hydrogels were used to formulate cyclosporin A suspensions for further evaluation. The results of the studies are shown in Table 5.

Initially, the inventors explored the possibility of heat-sterilizing a slurry of cyclosporin A of Form 1 (which converts to the amorphous form). This approach resulted in agglomeration of the drug and consequently, the formulation was not viable. Further studies, adding PVP to suppress the agglomeration of Form 1/amorphous form, also failed.

Since heat-sterilization of an aqueous suspension of cyclosporin did not appear to be viable, the inventors planned to prepare suspensions by aseptic technique, using pre-sterilize solid cyclosporin. Various solid cyclosporins (Forms 1, 2, and 3 and amorphous) were treated with gamma or e-beam irradiation. In all cases, significant loss of drug (3-9%) occurred (FIG. **2** and Table 1). Furthermore, the substantial loss of drug indicates that high levels of degradation products (around 3-9%) are generated in the irradiation-sterilized material. These impurities may have negative toxicological and/or regulatory implications; consequently, this approach to sterilization appears to be undesirable.

TABLE 1

Effect of Irradiation Sterilization on Cyclosporin (CsA) Drug Substance (solid)

Sterilization Mode	Form 1 CsA (Potency and Imp.)	Form 2 CsA (Potency and Imp.)	Form 3 CsA (Potency and Imp.)	Amorph. CsA (Potency and Imp.)
None	98.4% w/w Total Imp: 0.6%	94.6% w/w Total Imp: 0.6%	97.7% w/w Total Imp: 0.8%	96.5% w/w Total Imp: 0.7%
15 kGy Gamma	93.9% w/w % Rel. Change: 4.5% Total Imp: 1.7%	91.8% w/w % Rel. Change: 2.9% Total Imp: 1.8%	94.3% w/w % Rel. Change: 3.6% Total Imp: 1.3%	92.1% w/w % Rel. Change: 4.6% Total Imp: 1.4%
33 kGy Gamma	90.7% w/w % Rel. Change: 7.8% Total Imp: 2.8%	88.5% w/w % Rel. Change: 6.4% Total Imp: 2.4%	91.0% w/w % Rel. Change: 6.9% Total Imp: 2.3%	87.7% w/w % Rel. Change: 9.2% Total Imp: 2.3%
E-Beam	92.6% w/w % Rel. Change: 5.9% Total Imp: 1.5%	90.3% w/w % Rel. Change: 4.6% Total Imp: 1.7%	93.4% w/w % Rel. Change: 4.5% Total Imp: 1.6%	92.0% w/w % Rel. Change: 4.7% Total Imp: 1.3%

Subsequently, the inventors attempted to irradiate solid cyclosporin (Forms 1, 2, and 3 and amorphous), under the best conditions above, at cold temperatures. No significant improvement was noted with any of the Forms of cyclosporin (Table 2).

TABLE 2

Effect of E-Beam Sterilization of Cyclosporins under Cold Conditions

CsA Drug Substance Sample Treatment	CsA Potency for Control Sample	CsA Potency 15 kGy Gamma Treatment	CsA Potency 30 kGy Gamma Treatment	CsA Potency E-Beam 15 kGyTreatment
Dry Ice	99.2% w/w	96.7% w/w (% Rel. Change: 2.5%)	93.8% w/w (% Rel. Change: 5.4%)	93.8% w/w (% Rel. Change: 5.4%)
Cold Pack	96.5% w/w	93.0% w/w (% Rel. Change: 3.6%)	92.1% w/w (% Rel. Change: 4.6%)	93.2% w/w (% Rel. Change: 3.4%)

After it became apparent that irradiation of solid cyclosporins produced too much degradation, the inventors attempted to irradiate an aqueous suspension of cyclosporin, using hyaluronic acid as a suspending agent. This approach resulted in 4-10% degradation of the drug within the formulation.

TABLE 3

Effect of Sterilization by Irradiation on Aqueous Suspensions of Cyclosporin [CsA] using Hyaluronic Acid [HA] as a Suspending Agent, at Various Temperatures

Sterilization Treatment	CsA Potency for Control Sample	CsA Potency Post-Sterilization	% Relative Change in Potency
Cold Pack Control CsA Hydrogel Sample	103.2% w/w	Not Applicable	Not Applicable
CsA-HA Sample (Cold Pack) Treated with 15 kGy Gamma	103.2% w/w	98.9% w/w	4.2%
CsA-HA Sample (Cold Pack) Treated with 30 kGy Gamma	103.2% w/w	92.3% w/w	10.6%
CsA-HA Sample (Cold Pack) Treated with E-Beam (15 kGy)	103.2% w/w	92.8% w/w	10.1%

Finally, the inventor turned their focus on steam sterilization of slurries and full formulations of cyclosporins. Slurries of Form 1 (which converts to amorphous) agglomerate during heat-sterilization. Slurries of Form 3, while physically stable and more chemically stable than Form 1, degraded significantly during heat sterilization. But, to the inventors' surprise, slurries of Form 2 were both physically and chemically stable (Tables 4 and 5).

TABLE 4

Heat-Sterilization of Slurries of Cyclosporin (ScA) Form 2 (F-2) in Water

	CsA-F2 Slurry %	CsA-F3 Slurry %
Initial	96.86	101.41
120 C. 15 min	96.88	88.61
108 C. 60 min	106.69	71.72

TABLE 5

Physical Stabiltiy of Forms 2 and 3 Before and After Heat Sterilization

Formulation	Material	Spec.	D90	D50	D10	Conditions
A	CsA-F2	Slurry control	198.6313	116.8544	8.2711	Slurry control for steam sterilization study
A, autoclaved	CsA-F2	Autoclaved slurry	186.4431	99.902	7.0518	Autoclaved at 120 C. for 15 minutes
A, autoclaved	CsA-F2	Autoclaved slurry	195.603	112.532	9.209	Autoclaved at 108 C. for 60 minutes
B	CsA-F3	Slurry control	110.8281	63.3348	7.1711	Slurry control for steam sterilization study
B, autoclaved	CsA-F3	Autoclaved slurry	116.8761	67.523	12.1564	Autoclaved at 120 C. for 15 minutes
B, autoclaved	CsA-F3	Autoclaved slurry	115.556	65.3309	10.5518	Autoclaved at 108 C. for 60 minutes

Formulation	Material	Conditions	% potency compared to CsA Form 2 standard
A	CsA-F2	Control	96.9
A	CsA-F2	120° C., 15 min	96.9
A	CsA-F2	108° C., 60 min	106.7
B	CsA-F3	Control	101.4
B	CsA-F3	120° C., 15 min	88.6
B	CsA-F3	108° C., 60 min	71.7

Ocular Congestion

Parenterally-biocompatible suspending agents were identified by injecting sterile concentrated solutions into the subconjunctival space and evaluating the toxicological response. An injection of 100 ul of the following polymers in phosphate buffered saline was administered subconjunctivally to New Zealand white rabbits and observed for a period of seven days.

	type	name	source	Lot#	tech info	vendor	CoA	Grade	Alternative vendor	Grade
1	PVP	PVP K30	Sigma_Aldrich 81420-500G (or PSO R14247)	BCBB7859	Mw 40K (PSO: 5% in water, pH 3.6)	Sigma_Aldrich	yes		BASF	PHEUR/ USP/ NF/JP
2	PVP	PVP K90	Sigma_Aldrich 81440-250G	BCBB3954	Mw 360K	Sigma_Aldrich	yes		BASF	PHEUR/ USP/ NF/JP
3	PVP	PVP 10	Sigma-Aldrich PVP10-500G	050M0039	Mw 10K	Sigma_Aldrich	yes		BASF	PHEUR/ USP/ NF
4	HPMC	Hypromellose (tested to JP)	PSO PM# 1018 (R19424)	XB14012N11	Sigma H3785: 4000 cP, 2% in water	Dow Chemical	yes	USP/ PHEUR		
5	CMC	Carboxymethyl cellulose sodium	PSO R19716Q pending	96413					CMC from Ashland/ Aqualon is NF/USP,	
6	CMC	Carboxymethyl cellulose sodium	PSO R19717	96077						
7	Hydroxyethyl cellulose (HEC)	Natrosol (Type 250-HHX pharm)	Kevin Warner	F0854	Type 250-HHX pharm	Ashland			HEC from Ashland./ Aqualon is USP/EP,	
8	Acrylate/C10-30 Alkyl acrylate	Carbopol ETD 2020NF	Kevin Warner	EC742EK343	acrylate crosspolymer (Viscosity, 47-77K cP 0.5% wt at pH 7.5)	Lubrizol		USP/ NF		
9	Carbomer Interpolymer	Carbopol Ultrez 10 NF polymer	Kevin Warner	CC83RZG726	type A (Viscosity, 45-65K cP 0.5% wt at pH 7.5)	Lubrizol		USP/ NF		
10	Carbomer-Homopolymer	Carbopol 980 NF polymer	Kevin Warner	EC863CC625	type C (Viscosity, 40-60K cP 0.5% wt at pH 7.5)	Lubrizol		USP/ PHEUR/ JPE		
1	PVP	PVP K30	Sigma_Aldrich 81420-500G (or PSO R14247)	BCBB7859	Mw 40K (PSO: 5% in water, pH 3.6)	Sigma_Aldrich	yes		BASF	PHEUR/ USP/ NF/JP
2	PVP	PVP K90	Sigma_Aldrich 81440-250G	BCBB3954	Mw 360K	Sigma_Aldrich	yes		BASF	PHEUR/ USP/ NF/JP
3	PVP	PVP 10	Sigma-Aldrich PVP10-500G	050M0039	Mw 10K	Sigma_Aldrich	yes		BASF	PHEUR/ USP/ NF
4	HPMC	Hypromellose (tested to JP)	PSO PM# 1018 (R19424)	XB14012N11	Sigma H3785: 4000 cP, 2% in water	Dow Chemical	yes	USP/ PHEUR		
5	CMC	Carboxymethyl cellulose sodium	PSO R19716Q pending	96413					CMC from Ashland/ Aqualon is NF/USP,	
6	CMC	Carboxymethyl cellulose sodium	PSO R19717	96077						
7	Hydroxyethyl cellulose (HEC)	Natrosol (Type 250-HHX pharm)	Kevin Warner	F0854	Type 250-HHX pharm	Ashland			HEC from Ashland./ Aqualon is USP/EP,	
8	Acrylate/C10-30 Alkyl acrylate	Carbopol ETD 2020NF	Kevin Warner	EC742EK343	acrylate crosspolymer (Viscosity, 47-77K	Lubrizol		USP/ NF		

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	type	name	source	Lot#	tech info	vendor	CoA	Grade	Alternative vendor	Grade
9	Carbomer Interpolymer	Carbopol Ultrez 10 NF polymer	Kevin Warner	CC83RZG726	cP 0.5% wt at pH 7.5) type A (Viscosity, 45-65K cP 0.5% wt at pH 7.5)	Lubrizol		USP/ NF		
10	Carbomer-Homopolymer	Carbopol 980 NF polymer	Kevin Warner	EC863CC625	type C (Viscosity, 40-60K cP 0.5% wt at pH 7.5)	Lubrizol		USP/ PHEUR/ JPE		

2% Carbomer (Carbopol Ultrez 10NF, Lubrizol)
8% Carboxymethyl Cellulose (low viscosity CMC, Lubrizol)
6% Carboxymethyl Cellulose (high viscosity CMC, Lubrizol)
6% HEC (Ashland)
6% HPMC (Dow Chemical)
Juvederm Ultra (Allergan, Inc)
Pluronic F127 (BASF)
Polyvinyl pyrrolidone (PVP K90, BASF)

Gross ocular congestion was shown to resolve within 7 days for CMC, HEC, HPMC, Pluronic and PVP. Ocular discharge was shown to resolve within three days. Ocular discharge resolved within 3 days for all groups except one. Results of the experiment are provided in FIGS. **9-11**.

Impurity and Potency Analysis

The inventors prepared various formulations and evaluated their potency and purity, as well particle size distribution.

	CsA Crystal			Autoclave Conditions Temp (° C.)/Time	Particle size distribution		
	Lot #	Form	Excipient	(min.)	D90	D50	D10
	1	2	5% CMC	None	52.38	10.80	5.31
	2	2	5% CMC	121/10	18.02	11.55	5.74

	Composition					Impurities Analysis		
	CsA Particle			Potency (%)		Pre-Autoclave CsA Total	Post-Autoclave CsA Total	Absolute
Formulation	Size (μm)	CsA (%)	HEC (%)	No autoclave	Autoclave	Impurities (% a/a)	Impurities (% a/a)	Change (% a/a)
HEC-1	10	5	5	117.20%	115.70%	0.71%	0.69%	−0.02%
HEC-2	10	20	5	103.60%	116.60%	0.61%	0.61%	0.00%
HEC-3	10	5	2	116.40%	118.80%	0.78%	0.70%	−0.08%
HEC-4	10	20	1	124.50%	124.70%	0.73%	0.69%	−0.04%
HEC-5	25	5	5	126.70%	116.60%	0.58%	0.58%	0.00%
HEC-6	25	20	5	140.00%	147.40%	0.56%	0.56%	0.00%
HEC-7	25	5	2	137.50%	142.50%	0.63%	0.59%	−0.04%
HEC-8	25	20	2	129.50%	119.70%	0.56%	0.57%	0.01%
HEC-9	10	10	3	118.60%	111.70%	0.61%	0.62%	0.01%

	Composition				
	CsA Particle Size	CsA	PVP90	Potency (%)	
Formulation	(μm)	(%)	(%)	No autoclave	Autoclace
PVP-1	10	5	25	102.51	101.01
PVP-2	10	20	25	113.81	111.82
PVP-3	10	5	15	122.42	114.04
PVP-4	10	20	15	120.28	123.3
PVP-5	25	5	25	118.56	118.46
PVP-6	25	20	25	114.55	115.28
PVP-7	25	5	15	116.37	115.66
PVP-8	25	20	15	120.9	124.05
PVP-9	10	10	25	132.51	136.36
PVP-10	25	10	25	118.03	126.6

-continued

	CsA Crystal			Autoclave Conditions Temp (° C.)/Time	Particle size distribution		
	Lot #	Form	Excipient	(min.)	D90	D50	D10
	3	3	3% CMC	None	28.01	12.09	6.84
	4	3	3% CMC	121/10	20.31	11.27	6.56
	5	2	None	None	198.63	116.85	8.27
	6	2	None	120/15	186.44	99.90	7.05
	7	2	None	108/60	195.60	112.53	9.21
	8	3	None	None	110.83	63.33	7.17
	9	3	None	121/15	116.88	67.52	12.16
	10	3	None	108/60	115.56	65.33	10.55
	11	2	None	None	13.15	9.12	6.17
	12	2	None	121/15	14.15	9.12	6.42

-continued

Lot #	CsA Crystal Form	Excipient	Autoclave Conditions Temp (° C.)/Time (min.)	Particle size distribution D90	D50	D10
13	2	None	None	14.14	9.66	6.44
14	2	None	121/15	14.30	9.37	5.95

TABLE 5

Key F2 Formulation Properties of Evaluated Polymers

	Syringeability	Autoclavability (121 C., 15 min.)	In-vivo tolerability (1 wk sub-conj.)	Settling	CSA-F2 Potency
Carbopol	Max. conc. 4% w/22 G	No visible change	Poorly tolerated (congestion)	na	na
Carboxymethyl Cellulose (CMC) medium viscosity	Max. conc. 9% w/22 G	No visible change	Poorly tolerated (congestion)	na	na
Carboxymethyl Cellulose (CMC) high viscosity	Max. conc. 6% w/22 G	No visible change	Poorly tolerated (congestion)	na	na
Hydroxyethyl Cellulose (HEC)	Max. conc. 6% w/22 G	No visible change	Well tolerated. Slight congestion compared to saline.	No settling in comparison with BDP gel under same conditions	No loss in potency post-autoclave
Hydroxypropyl Methyl Cellulose (HPMC)	Max. conc. 7% w/22 G	Full formation visibly separates with moist heat sterilization	Well tolerated. Comparable to saline.	na	na
Juvederm Ultra	30 g, as formulated by manufacturer	Pre-sterilized by manufacturer	poorly tolerated (swelling at 1 week)	na	na
Pluronic F127	Max. cone. 40% w/22 G	Placebo visibly separates with moist heat sterilization	Tolerated. Slight congestion and discharge compared to saline.	na	na
Polyvinylpyrrolidone K90 (PVPK90)	Max. conc. 27% w/22 G	No visible change	Ok	Some settling with syringeable concentrations (but acceptable)	No loss in potency post-autoclave

What is claimed is:

1. A method of making a formulation of cyclosporin A, the method comprising the steps of

a) dissolving cyclosporin A form 2 in solution;

b) autoclaving the solution; and

c) adding a vehicle selected from the group consisting of carboxymethyl cellulose, hydroxypropyl methyl cellulose, hydroxyethyl cellulose, hyaluronic acid, polyvinylpyrrolidone, crosslinked polyacrylic acid polymers, and copolymers based on ethylene oxide and propylene oxide.

2. A method of making a formulation of cyclosporin A, the method comprising the steps of

a) dissolving in solution a vehicle selected from the group consisting of carboxymethyl cellulose, hydroxypropyl methyl cellulose, hydroxyethyl cellulose, hyaluronic acid, polyvinylpyrrolidone, crosslinked polyacrylic acid polymers, and copolymers based on ethylene oxide and propylene oxide;

b) adding to the solution cyclosporin A Form 2; and

c) autoclaving the resulting mixture.

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